Genomic DNA Extraction

Requirements:

—TransGen EasyPure® Bacteria Genomic DNA Kit
—100% ethanol
—70% ethanol (for Gram-positive bacterium)
—Glass bead (for Actinobacillus)
—Lysozyme (produced by BBI Life Sciences®)
—RNase A (produced by Takara®)
—Proteinase K (produced by Takara®)
—Microcentrifuge capable of at least 13,000 x g
—Nuclease-free 1.5mL microcentrifuge tubes
—Sterile deionized water
—Electric dry oven at 65°C
—Water bath at 55°C and 65°C

Before Starting:

—Add 15mL 100% ethanol into BB11 if there's no mark on the bottle and store at room temperature
—Add 48mL 100% ethanol into WB11 if there's no mark on the bottle and store at room temperature
—Weight 4mg lysozyme in a microcentrifuge tube, and add 200µL RB11, store at 4°C
—Heat sterile deionized water to 65°C using water bath

Protocol:

1. Pellet 1.5mL bacteria in a clean 1.5mL microcentrifuge tube by centrifugation at 12,000 x g for 1 minute at room temperature. Decant or aspirate medium and discard.
2. Add 200µL RB11/lysozyme, pipet up and down to mix thoroughly. Incubate at 37°C for 60 minutes.
3. Centrifuge at 10,000 x g for 1 minute. Discard the liquid.
   Note: For Gram-positive bacterium, a re-suspension using 70% ethanol before this step is needed. For Actinobacillus, it's necessary to scatter the hyphostroma.
4. Add 100µL LB11 and 20µL Proteinase K, pipet up and down to mix thoroughly.
5. Incubate at 55°C for 15 minutes, shake the tube every 5 minutes.
   Note: If the liquid is not limpid, another 15 minutes incubation may be necessary.
6. Add 20µL RNase A, mix thoroughly, and incubate at room temperature for 2 minutes.
7. Add 400µL BB11, vortex 30 seconds.
8. Transfer 700µL liquid from Step 7 into a Genomic Spin Column.
9. Centrifuge at 12,000 x g for 30 seconds, discard the filtrate and reuse the collection tube.
10. Repeat Steps 8-9 until all liquid has been transferred to the Genomic Spin Column.
11. Add 500µL CB11 into the Genomic Spin Column, centrifuge at 12,000 x g for 30 seconds, discard the filtrate and reuse the collection tube.
12. Repeat Step 11 once.

13. Add 500µL WB11 into the Genomic Spin Column, centrifuge at 12,000 x g for 30 seconds, discard the filtrate and reuse the collection tube.
14. Repeat Step 13 once.
15. Centrifuge the empty Genomic Spin Column for 2 minutes at 12,000 x g to dry the column matrix.
16. Transfer the column to a clean 1.5mL microcentrifuge tube. Open the lid and put it in the electric dry oven for 10 minutes to volatilize alcohol.
17. Add 75µL sterile deionized water into the column.
18. Let sit at room temperature for 2 minutes.
19. Centrifuge at 12,000 x g for 1 minute.
20. Repeat Steps 17-19 once.
21. Store DNA at -20 °C.